



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/62, 15/13, C07K 19/00, 16/30, A61K 47/42, 39/395, G01N 33/577, 33/574, C07K 16/00, 16/46 // 14/21		A1	(11) International Publication Number: WO 96/13594 (43) International Publication Date: 9 May 1996 (09.05.96)									
<p>(21) International Application Number: PCT/US95/13811</p> <p>(22) International Filing Date: 26 October 1995 (26.10.95)</p> <p>(30) Priority Data:</p> <table> <tr> <td>08/331,396</td> <td>28 October 1994 (28.10.94)</td> <td>US</td> </tr> <tr> <td>08/331,397</td> <td>28 October 1994 (28.10.94)</td> <td>US</td> </tr> <tr> <td>08/331,398</td> <td>28 October 1994 (28.10.94)</td> <td>US</td> </tr> </table> <p>(71) Applicant: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Box OTT, Bethesda, MD 20892 (US).</p>		08/331,396	28 October 1994 (28.10.94)	US	08/331,397	28 October 1994 (28.10.94)	US	08/331,398	28 October 1994 (28.10.94)	US	<p>(72) Inventors: PASTAN, Ira; 11710 Beall Mountain Road, Potomac, MD 20854 (US). BENHAR, Itai; 341 Congressional Lane, Rockville, MD 20852 (US). PADLAN, Eduardo, A.; 4006 Simms Drive, Kensington, MD 20895 (US). JUNG, Sun-Hee; 259 Congressional Lane #206, Rockville, MD 20852 (US). LEE, Byungkook; 10711 Sandy Landing Road, Potomac, MD 20854 (US). WILLINGHAM, Mark; 108 Kimberwicke Drive, Summerville, SC 29483 (US). FITZGERALD, David; 1202 Azalea Drive, Rockville, MD 20850 (US). BRINKMANN, Ulrich; 4404 Everett Street, Kensington, MD 20895 (US). PAI, Lee; 34 Tivoli Lake Street, Silver Spring, MD 20906 (US).</p> <p>(74) Agents: WEBER, Ellen, Lauver et al.; Townsend and Townsend and Crew, Steuart Street Tower, One Market, San Francisco, CA 94105-1492 (US).</p> <p>(81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
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<p>(54) Title: TUMOR-SPECIFIC ANTIBODY FRAGMENTS, FUSION PROTEINS, AND USES THEREOF</p> <p>(57) Abstract</p> <p>This invention provides for recombinant single chain antibodies capable of specifically binding to a Lewis^Y-related carbohydrate antigen and fusion proteins comprising these antibodies. More particularly, the invention provides for single chain Fv (ScFv) regions of the monoclonal antibodies B1, B3, and B5, humanized single chain Fv regions of B1, B3, and B5, and fusion proteins comprising these scFv regions. The invention also provides for a number of stabilizing mutations of the Lewis^Y-binding monoclonal antibody B3. In addition, the invention provides for methods of detecting cells bearing a Lewis^Y antigen in a patient and for methods of killing or inhibiting the growth of cells bearing a Lewis^Y antigen in a patient. The invention also provides for a method of improving the binding affinity of antibodies lacking a serine at position 95 of the V_H region that involves mutating position 95 to a serine.</p>												

*(Referred to in PCT Gazette No. 14/2000, Section II)

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1 72. A single chain fusion protein comprising:

- 2 a) a humanized Fv region of both the light and heavy chains of
3 an antibody selected from the group consisting of B1, B3 and B5; and
4 b) an effector molecule;

5 wherein the humanized Fv region and the effector molecule are recombinantly fused to
6 form a single molecule.

1 73. The fusion protein of claim 72, wherein the Fv region of said
2 protein comprises a humanized variable heavy chain having the amino acid sequence
3 designated HumB3V_H in Figure 11.

1 74. The fusion protein of claim 72, wherein the Fv region of said
2 protein comprises a humanized variable heavy chain having the amino acid sequence
3 designated HumB3V_L in Figure 11.

1 75. The fusion protein of claim 72, wherein the Fv region of said
2 protein comprises a humanized variable heavy chain having the amino acid sequence
3 designated HumB3V_H in Figure 11 and a humanized variable light chain having the
4 amino acid sequence designated HumB3V_L in Figure 11.

1 76. The fusion protein of claim 72, wherein the Fv region of said
2 protein comprises a humanized variable heavy chain having the amino acid sequence
3 designated HumB3V_H in Figure 11 with the exception that the sequence has an arginine
4 at a position in said variable heavy chain designated as 82b in Figure 11 replaced serine
5 with arginine at the position in the variable heavy chain designated as 82b in Figure 11.

1 77. The fusion protein of claim 72, wherein said effector molecule is a
2 *Pseudomonas* exotoxin.

1 78. The fusion protein of claim 77, wherein said *Pseudomonas* exotoxin
2 is PE38, PE40, PE38KDEL and PE38REDL.

1 79. A recombinant DNA molecule that encodes a humanized Fv region
2 of both the light and heavy chains of an antibody wherein said antibody is a monoclonal
3 antibody selected from the group consisting of B1, B3 and B5.

1 80. The recombinant DNA molecule of claim 79, wherein said DNA
2 sequence encodes an Fv region comprising a humanized variable heavy chain having the
3 amino acid sequence designated HumB3V_H in Figure 11.

1 81. The recombinant DNA molecule of claim 79, wherein said DNA
2 sequence encodes an Fv region comprising a humanized variable light chain having the
3 amino acid sequence designated HumB3V_L in Figure 11.

1 82. The recombinant DNA molecule of claim 79, wherein said DNA
2 sequence encodes an Fv region comprising a humanized variable heavy chain having the
3 amino acid sequence designated HumB3V_H in Figure 11a and a humanized variable light
4 chain having the amino acid sequence designated HumB3V_L in Figure 11.

1 83. The recombinant DNA molecule of claim 79, wherein said DNA
2 sequence encodes an Fv region comprising a humanized variable heavy chain having the
3 amino acid sequence designated HumB3V_H in Figure 11A with the exception that there is
4 an arginine at a position in said variable heavy chain designated as 82b in Figure 11.

1 84. A recombinantly produced protein comprising a humanized Fv
2 region of both a light and a heavy chain of an antibody wherein said antibody is a
3 monoclonal antibody selected from the group consisting of B1, B3 and B5.

1 85. The protein of claim 84, wherein said protein comprises a
2 humanized variable heavy chain having the amino acid sequence designated HumB3V_H in
3 Figure 11.

1 86. The protein of claim 84, wherein said protein comprises a
2 humanized variable heavy chain having the amino acid sequence designated HumB3V_L in
3 Figure 11.

1 87. The protein of claim 84, wherein said protein comprises a
2 humanized variable heavy chain having the amino acid sequence designated HumB3V_H in
3 Figure 11 and a humanized variable light chain having the amino acid sequence
4 designated HumB3V_L in Figure 11.

1 88. The protein of claim 84, wherein said protein comprises a
2 humanized variable heavy chain having the amino acid sequence designated HumB3V_H in
3 Figure 11 with the exception that there is a variable heavy chain is back arginine at a
4 position in the variable heavy chain designated as 82b in Figure 11.

1 89. A pharmaceutical composition comprising a recombinantly
2 produced single chain fusion protein in a concentration sufficient to inhibit tumor growth,
3 together with a pharmaceutically acceptable carrier wherein said fusion protein
4 comprises:

5 a) a humanized Fv region of both a light and a heavy chain of an
6 antibody wherein said antibody is a monoclonal antibody selected from the group
7 consisting of B1, B3 and B5; and

8 b) an effector molecule;

9 wherein both of said Fv regions and said effector molecule are recombinantly fused to
10 form a single molecule that has the binding specificity of a monoclonal antibody selected
11 from the group consisting of B1, B3 and B5.

1 90. The composition of claim 89, wherein said effector molecule is a
2 *Pseudomonas* exotoxin.

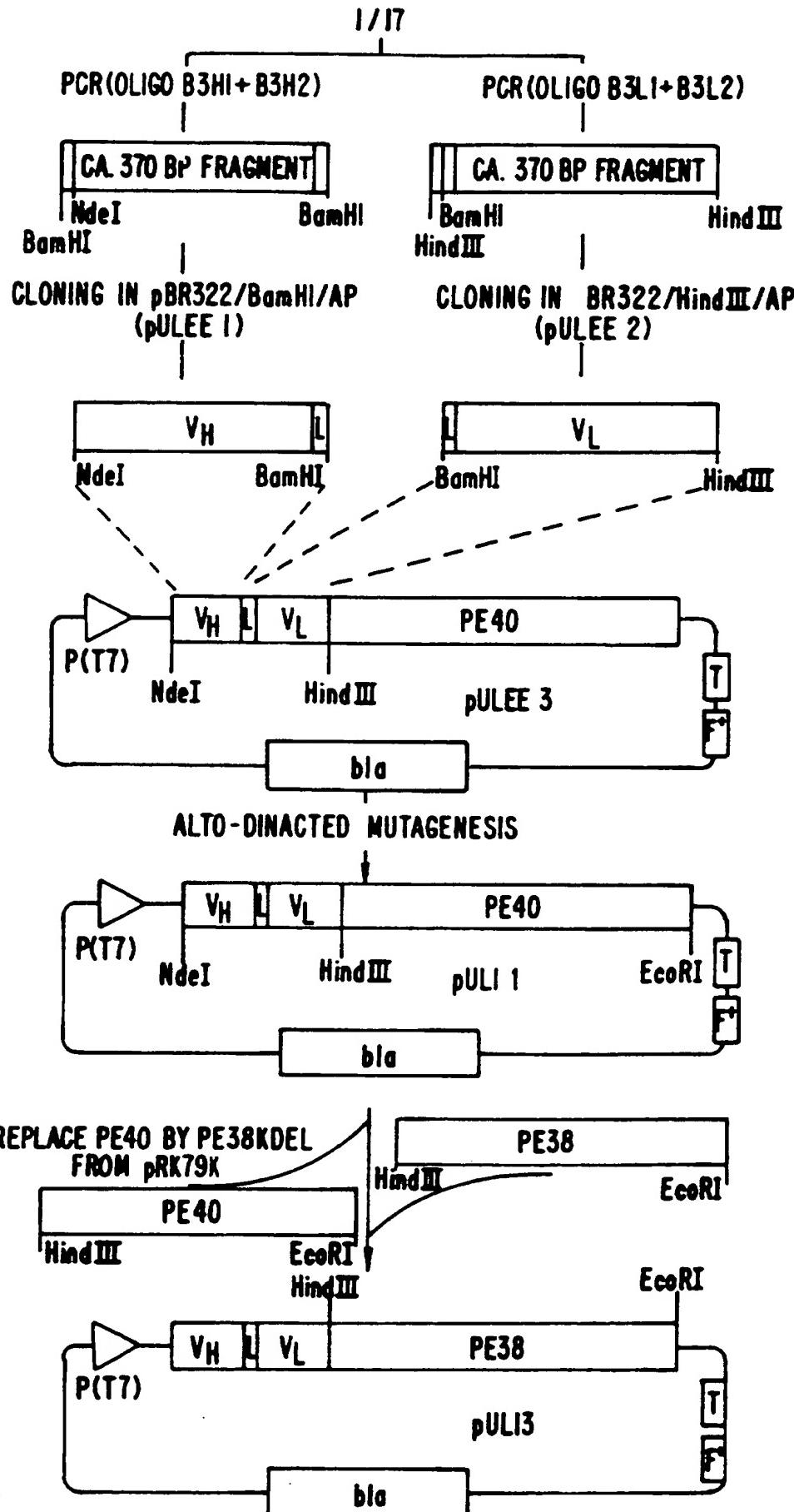
1 91. The composition of claim 90, wherein said effector molecule is
2 selected from the group consisting of PE38, PE40, PE38KDEL, PE38REDL.

1 92. The composition of claim 89, wherein said humanized Fv region is
2 a humanized B3(Fv) region.

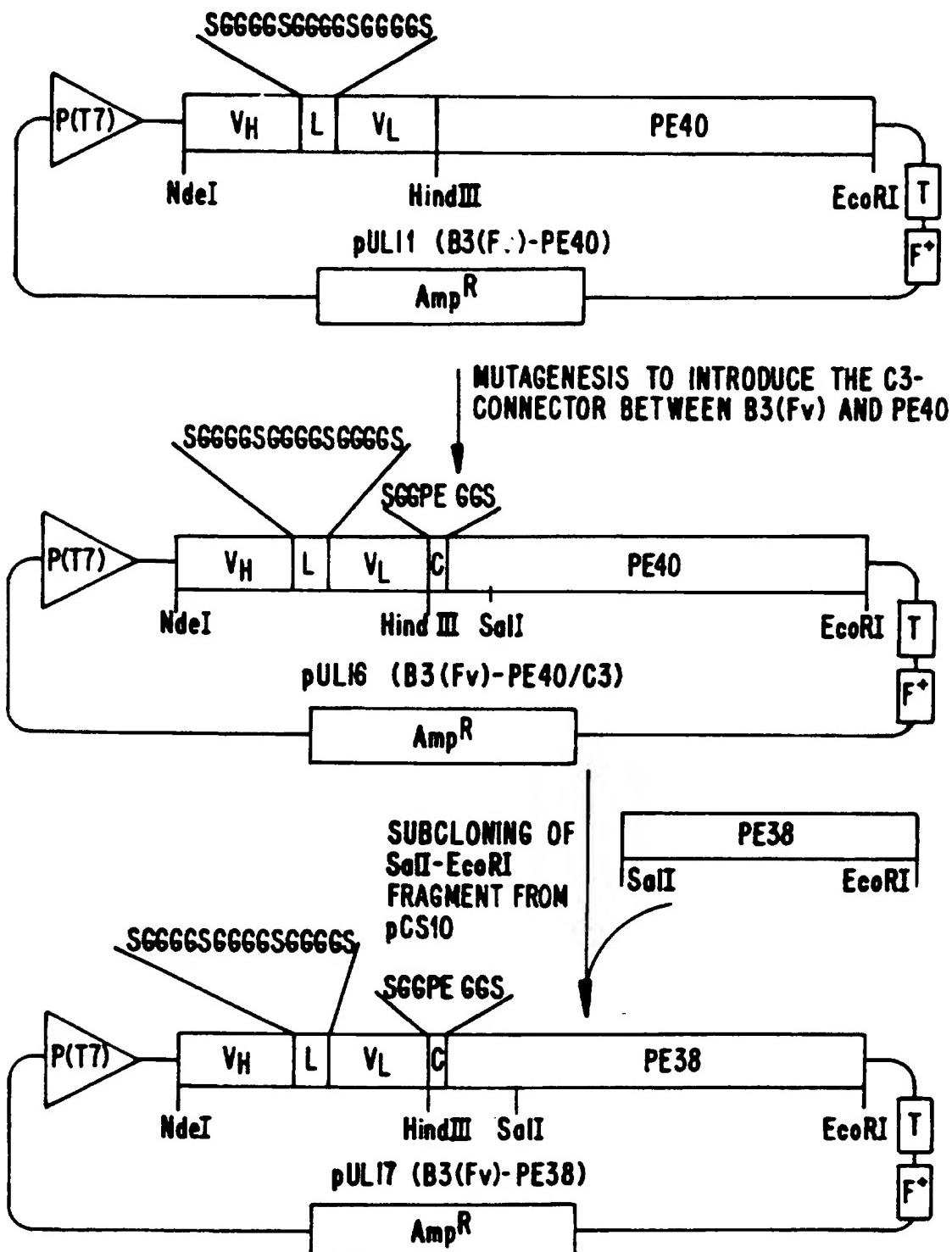
1 93. A method of detecting the presence or absence of a cell bearing a
2 Lewis^y carbohydrate antigen in a patient, said method comprising the steps of:

- 3 a) removing a tissue or fluid sample from said patient;
- 4 b) adding an antibody to said sample wherein said antibody
- 5 comprises:
- 6 i) a humanized Fv region of both a light and a heavy chain
- 7 of an antibody wherein said antibody is a monoclonal antibody selected from the group
- 8 consisting of B1, B3 and B5; and
- 9 ii) an effector molecule;
- 10 further wherein said Fv regions are recombinantly fused to
- 11 form a single molecule that has the binding specificity of a monoclonal antibody selected
- 12 from the group consisting of B1, B3 and B5; and
- 13 c) detecting for the presence or absence of a binding complex
- 14 between the antibody and the antigen.

- 1 94. A method of killing or inhibiting the growth of cells bearing a
- 2 Lewis^X antigen in a patient, said method comprising administering to the patient a
- 3 pharmaceutical composition in an amount sufficient to kill or inhibit the growth of said
- 4 cells, said composition comprising:
- 5 a) a humanized Fv region of both a light and a heavy chain of an
- 6 antibody wherein said antibody is a monoclonal antibody selected from the group
- 7 consisting of B1, B3 and B5; and
- 8 b) an effector molecule;
- 9 wherein both of said Fv regions and said effector molecule are recombinantly fused to
- 10 form a single molecule that has the binding specificity of a monoclonal antibody selected
- 11 from the group consisting of B1, B3 and B5.

**FIG. 1A.**

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**FIG. 1B.**

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NdeI |----Fv HEAVY CHAIN--

1 TTTAACTTAAGAAGGAGATATACTATGGATGTGAAGCTGGTGGAGTCT 50
 SD M D V K L V E S
 E V K L V E S

----->

51 GGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAAC 100
 G G G L V Q P G G S L K L S C A T
 G G G L V Q P G G S L

101 CTCTGGATTCACTTCAGTGACTATTACATGTATTGGGTTGCCAGACTC 150
 S G F T F S D Y Y M Y W V R Q T P

151 CAGAGAAGAGGCTGGAGTGGTCGCATACATTAGTAATGATGATAGTTCC 200
 E K R L E W V A Y I S N D D S S

201 GCCGCTTATTCAAGACACTGTAAAGGGCCGGTTCACCATCTCCAGAGACAA 250
 A A Y S D T V K G R F T I S R D N

251 TGCCAGGAACACCCCTCACCTGCAAATGAGCCGTCTGAAGTCTGAGGACA 300
 A R N T L Y L Q M S R L K S E D T

301 CAGCCATATATTCCGTGCAAGAGGACTGGCCTGGGGAGCCTGGTTGCT 350
 A I Y S C A R G L A W G A W F A

BamHI

351 TACTGGGCCAAGGGACTCTGGTCACTGTCTCCTCAGGCAGGCGGATC 400
 Y W G Q G T L V T V S S G G G G S
 <----Fv HEAVY CHAIN--|-----LINKER-

-----LINKER-----|---Fv LIGHT CHAIN---

401 CGGTGGTGGCGGATCTGGAGGTGGCGGAAGCGATGTGCTGATGACCCAGT 450
 G G G G S G G G S D V L M T Q S
 D V L M T Q S

-----Fv LIGHT CHAIN----->

451 CTCCATTGAGTTACCTGTCAGTCTGGAGATCAAGCCTCCATCTTGC 500
 P L S L P V S L G D Q A S I S C
 P L S L P V S L G ? Q

501 AGATCTAGTCAGATCATTGTACATAGTAATGGAAACACCTATTTAGAATG 550
 R S S Q I I V M S N G N T Y L E W

551 GTACCTGCAGAAACCAGGCCAGTCTCAAAGCTCCTGATCTACAAAGTTT 600
 Y L Q K P G Q S P K L L I Y K V S

FIG. 2A-1.

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601 CCAACCGATTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGG 650
N R F S G V P D R F S G S G S G S G

651 ACAGATTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGT 700
T D F T L K I S R V E A E D L G V

701 TTATTACTGCTTCAAGGTTCACATGTTCCATTCACGTTGGCTCGGGGA 750
Y Y C F Q G S N V P F T F G S G T

HindIII

751 CAAAGCTGGAAATTAAAGCTTT..... 772
K L E I K A F -> PE40

FIG. 2A-2.

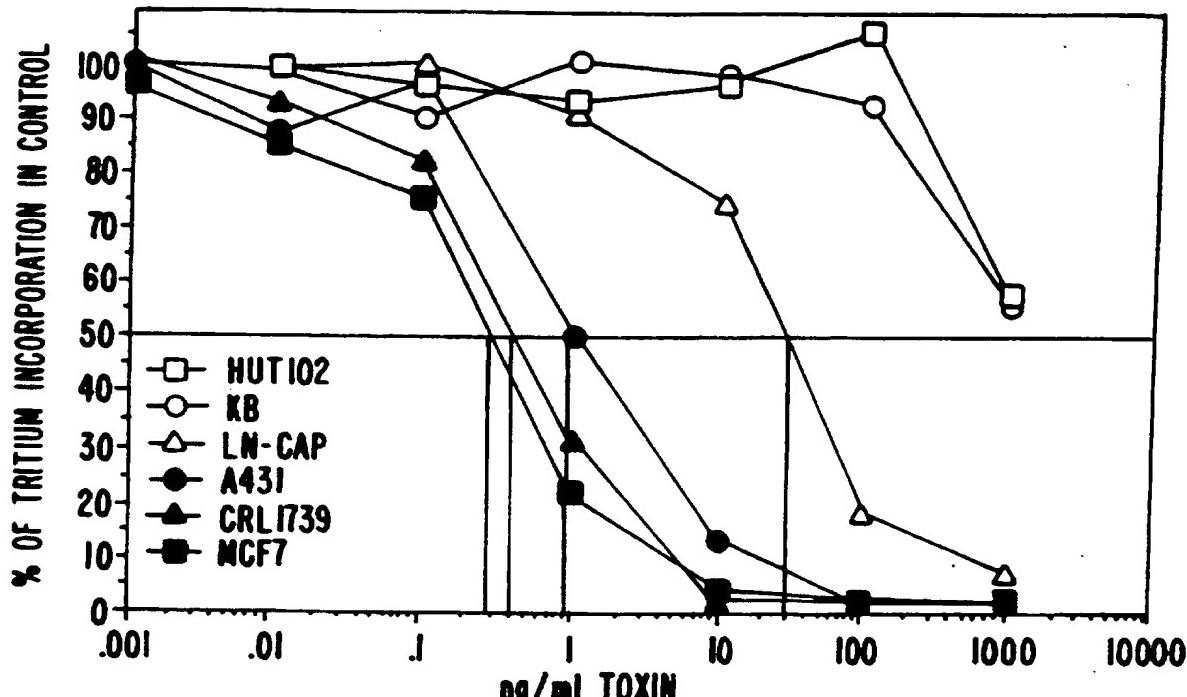
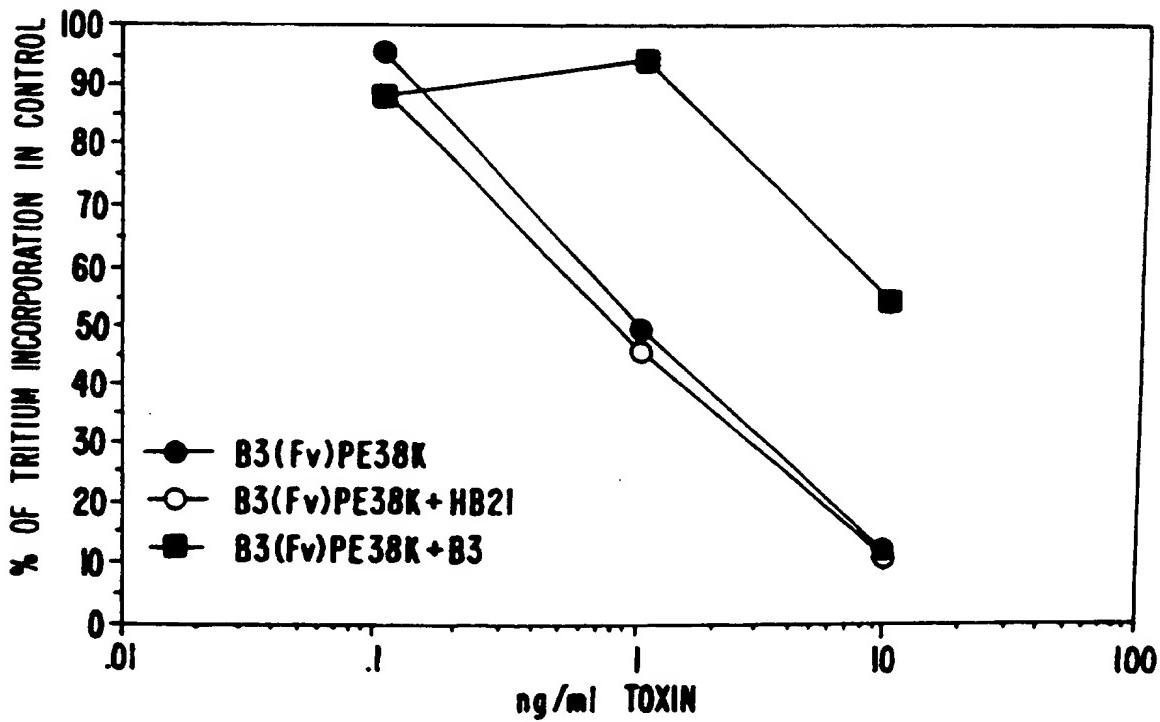
721 CACATGTTCCATTCACGTTGGCTCGGGGACAAAGCTGGAAATTAAATAA 770
H V P F T F G S G T K L E I K *

EcoRI

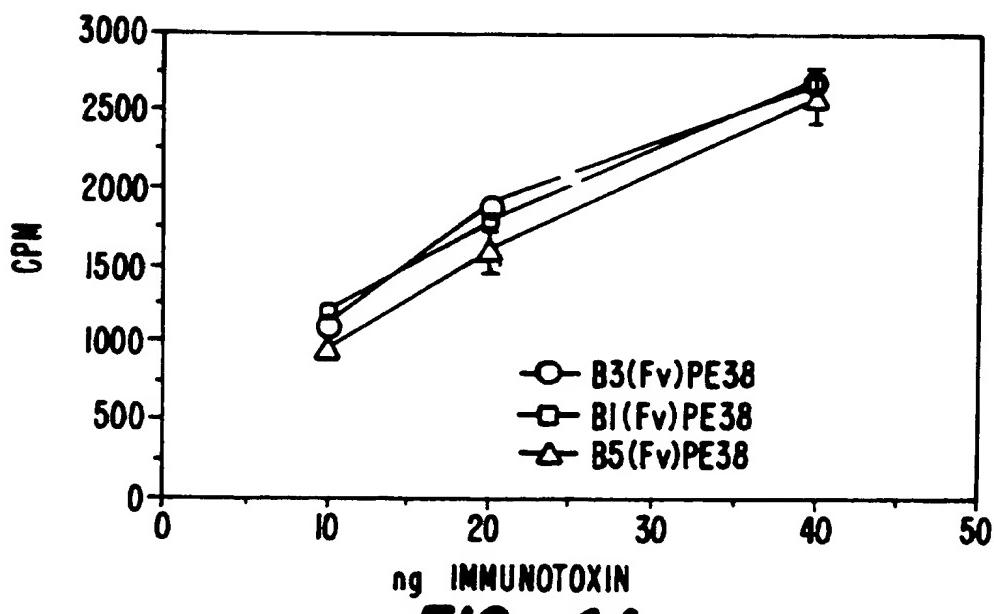
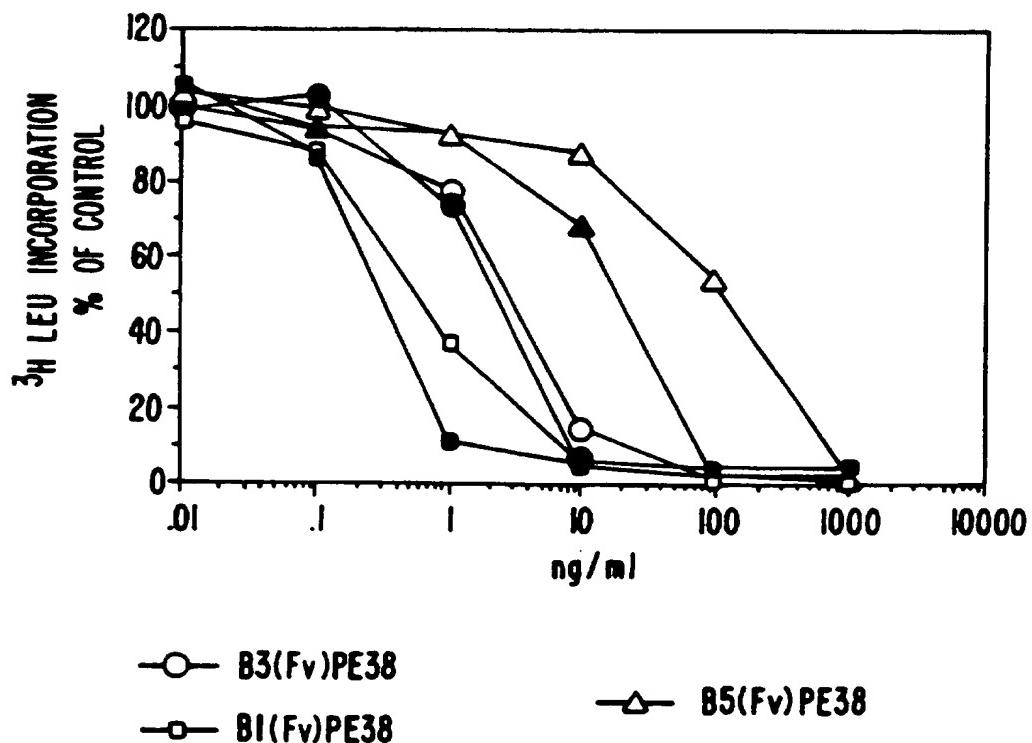
771 TGAATTCC.. 779
* -> TERM

FIG. 2B.

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**FIG. 3A.****FIG. 3B.**

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**FIG. 4A.****FIG. 4B.**

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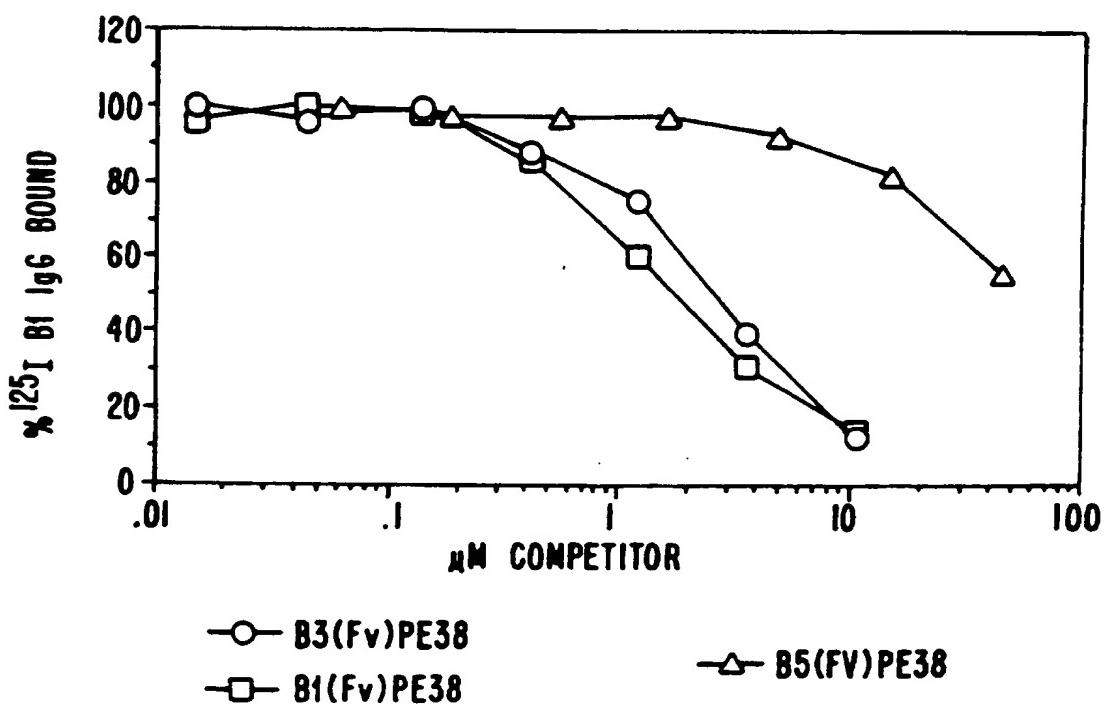
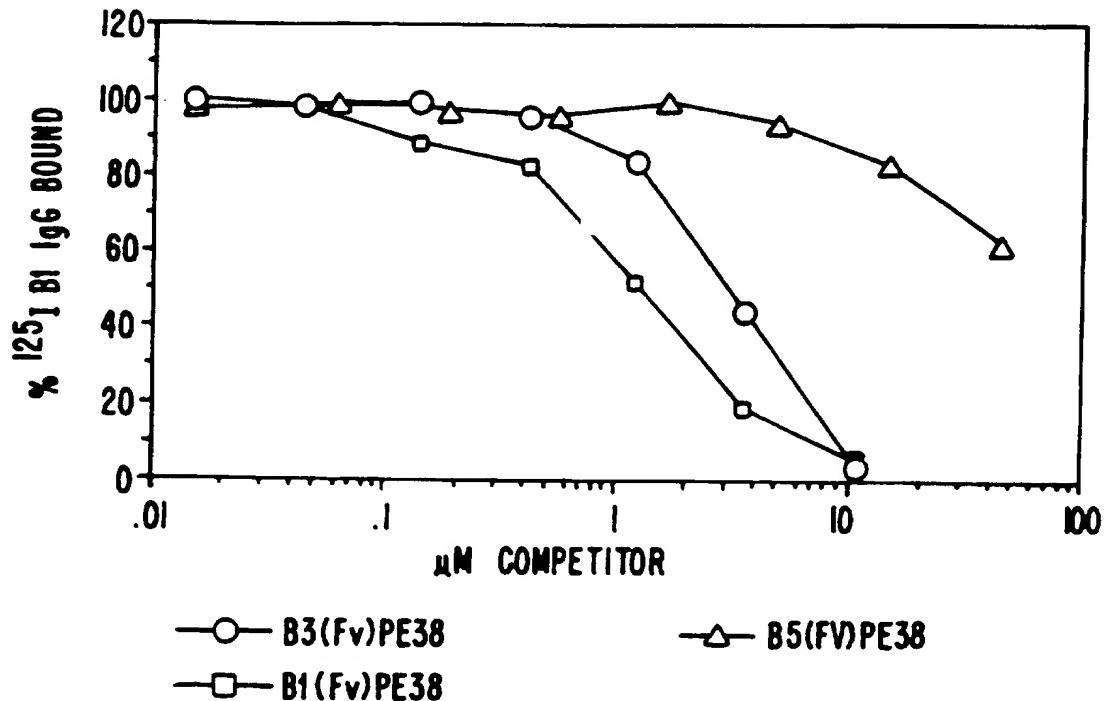
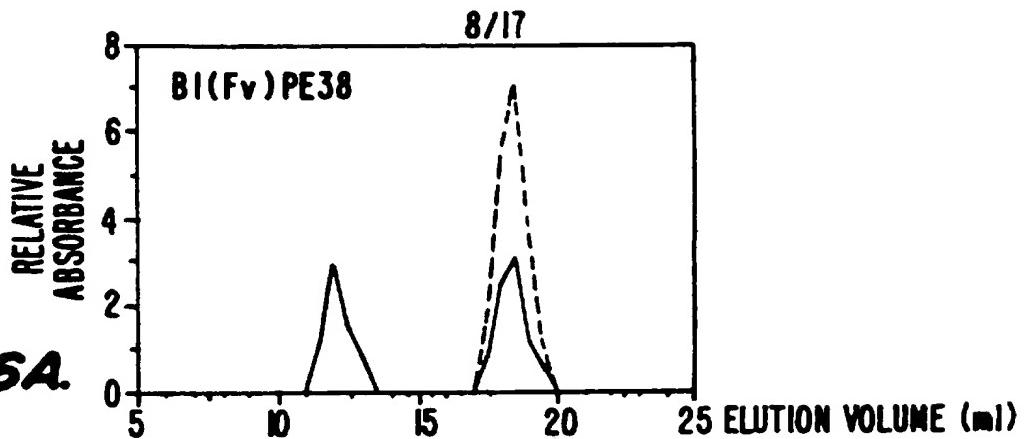
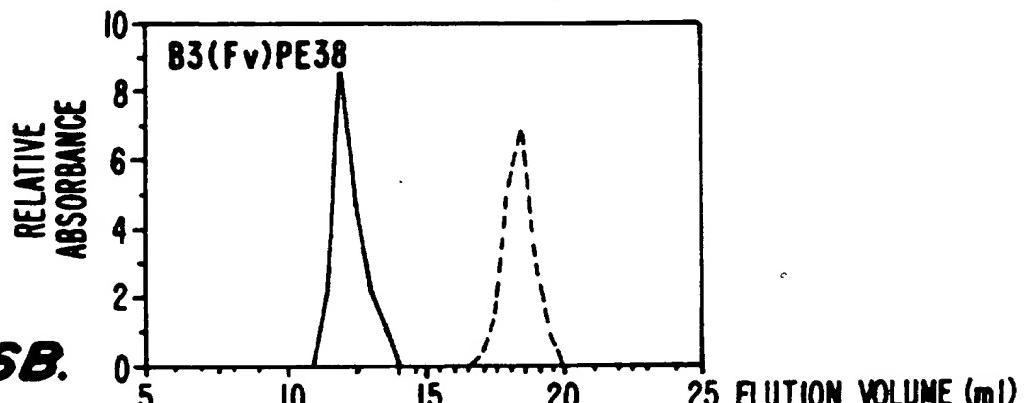
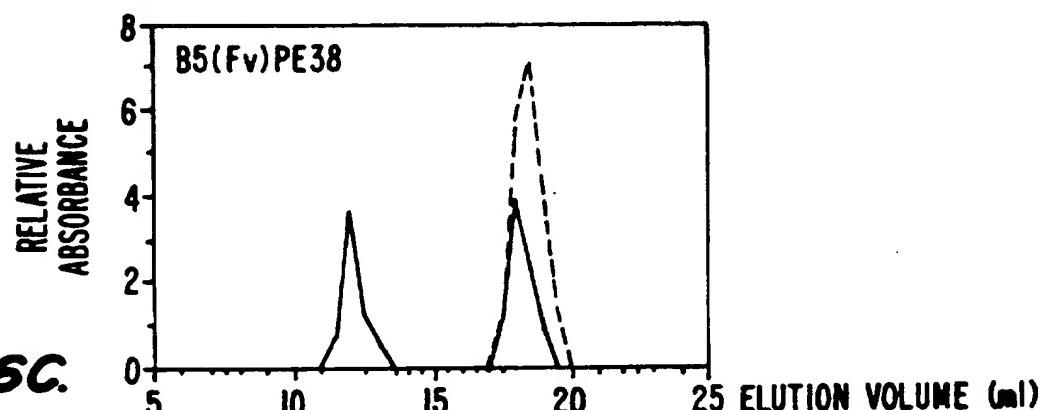
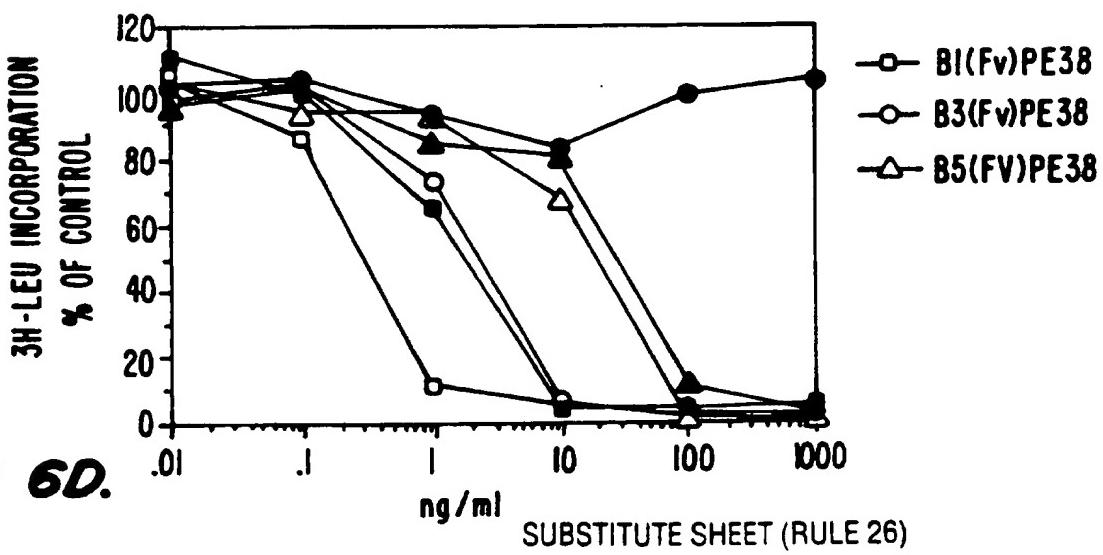
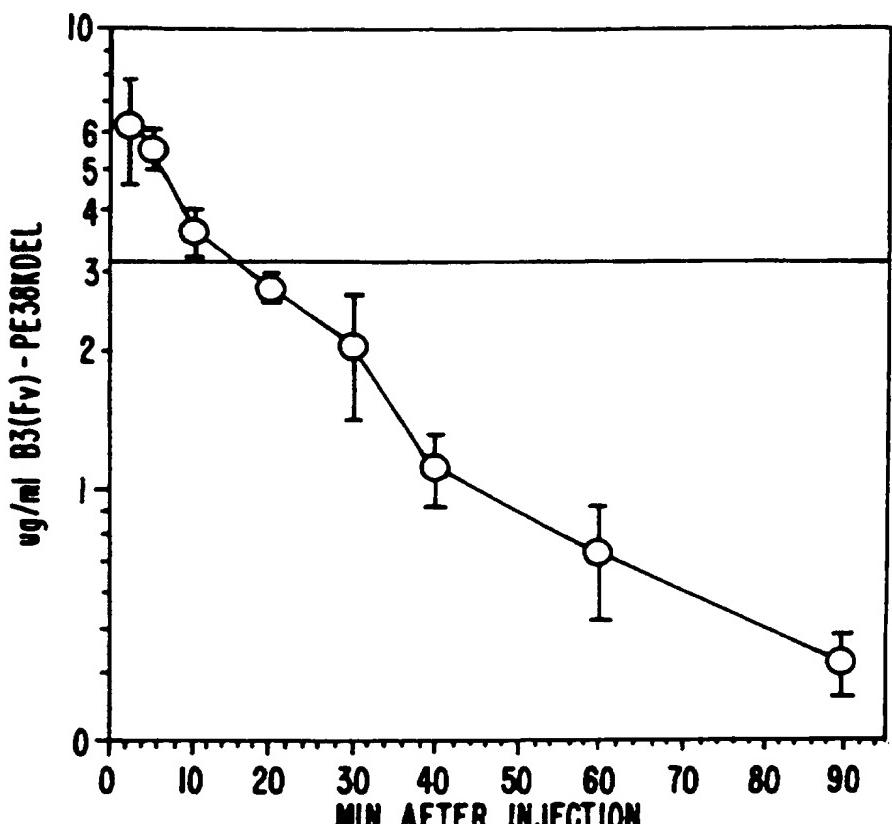
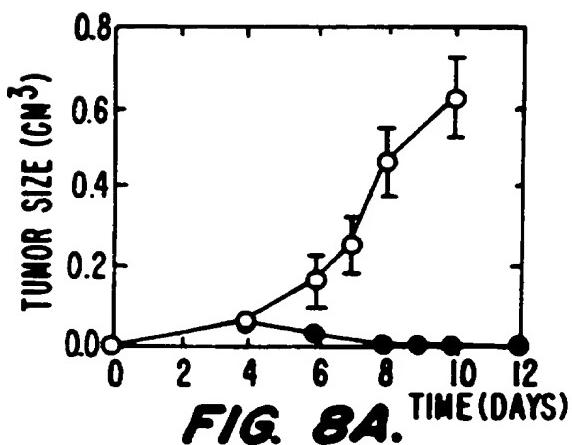
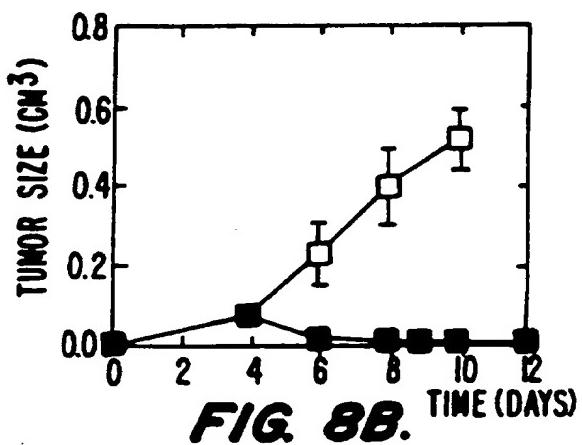
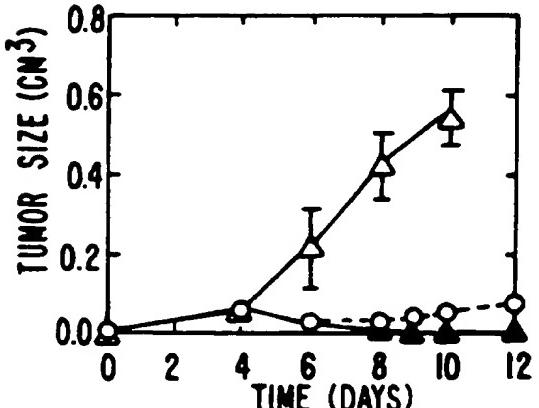
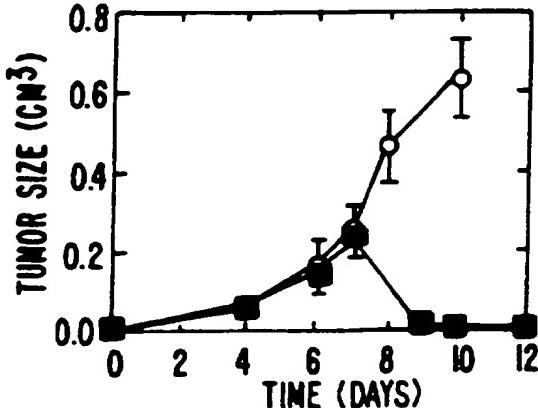
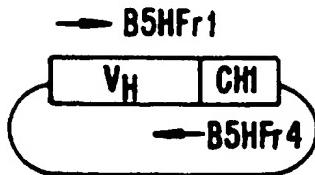
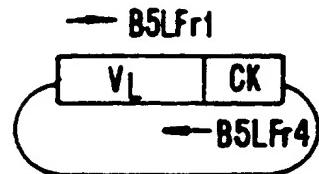


FIG. 6A.**FIG. 6B.****FIG. 6C.****FIG. 6D.**

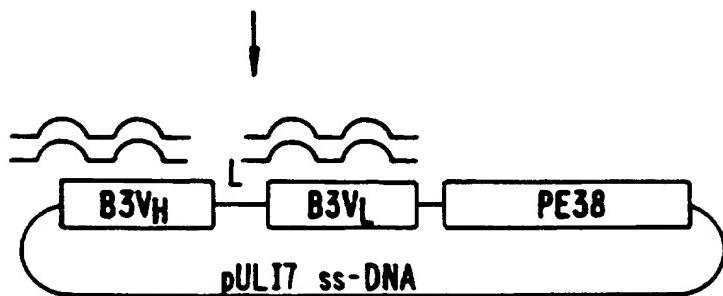
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**FIG. 7.****FIG. 8A.****FIG. 8B.****FIG. 8C.****FIG. 8D.**

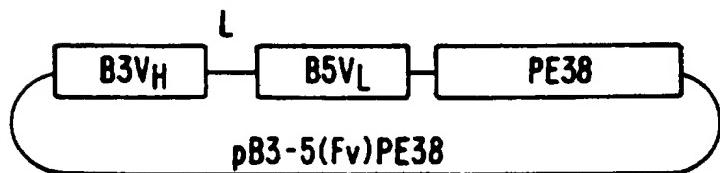
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pCR-B5V_HpCR-B5V_L

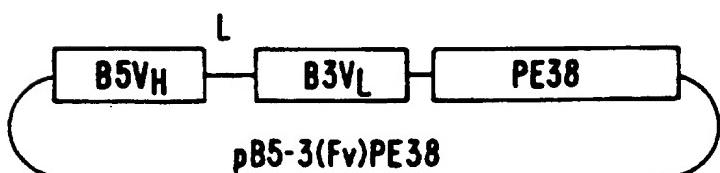
PCR AMPLIFY VARIABLE
SEGMENTS, ANNEAL PCR
PRODUCTS SEPARATELY OR
COMBINED TO THE TEMPLATE



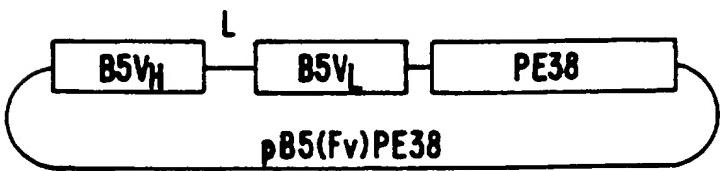
| EXTEND AND LIGATE
| TRANSFROM *E.coli* DH5 α



OR



OR

**FIG. 9.**

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FIG. 10A.

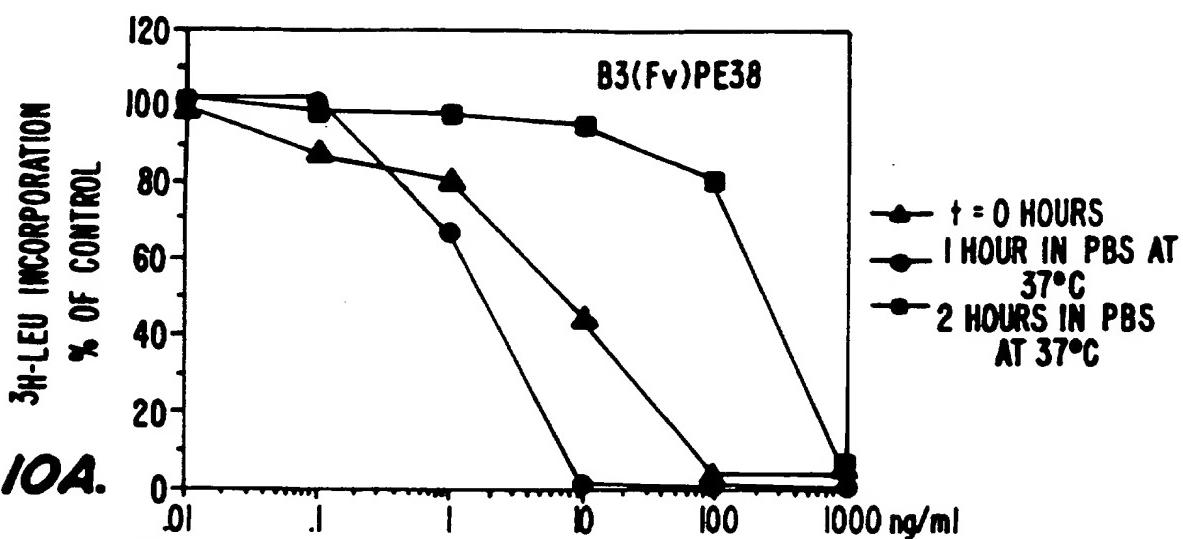


FIG. 10B.

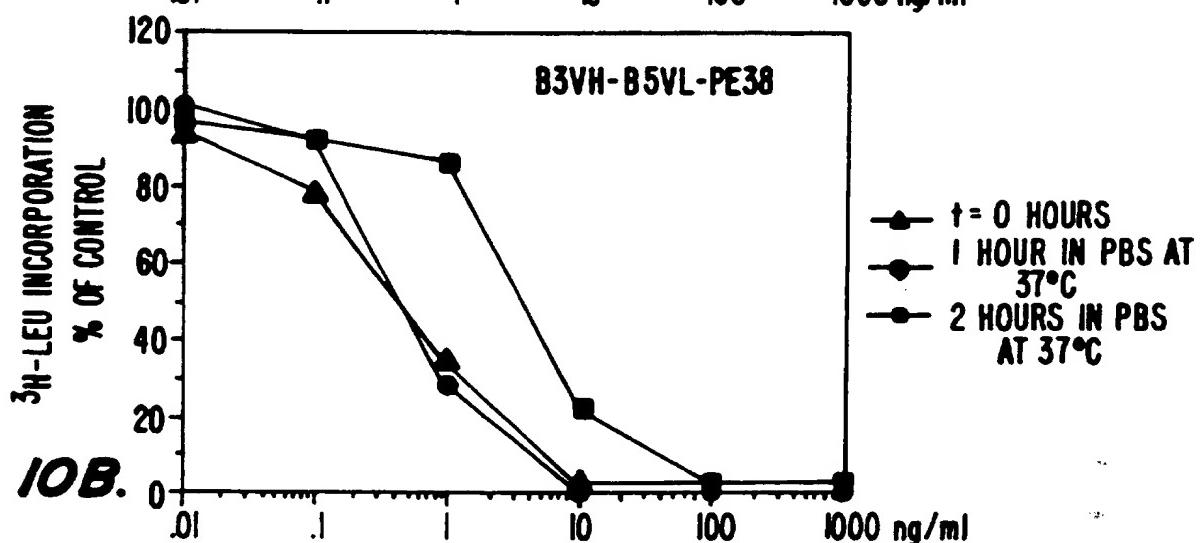
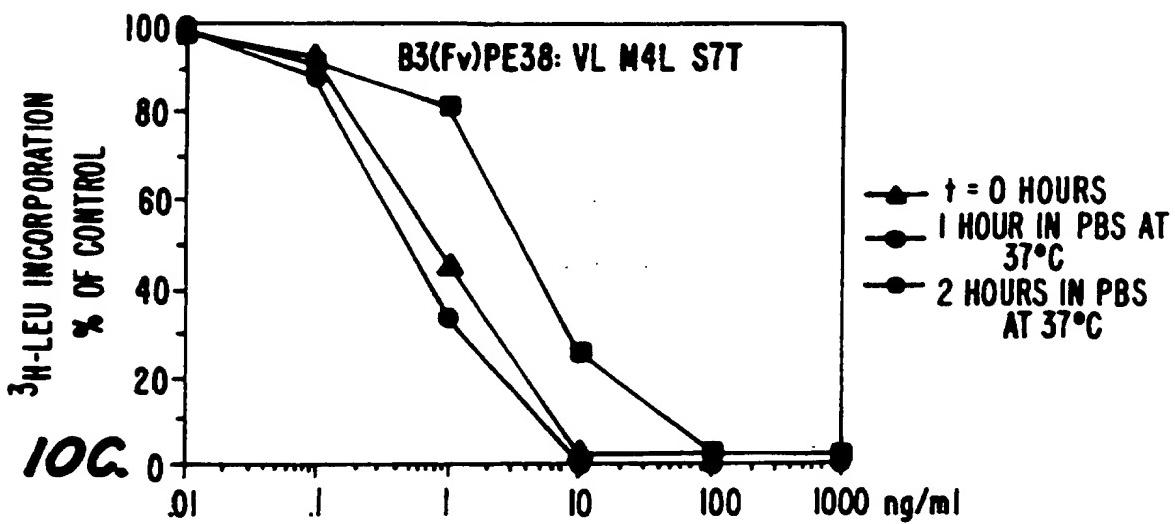


FIG. 10C.



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11 16 | | | | CDR1 | | 40 42 44
 | | | | |
 DVKLVESGGGLVQPGGSLKLSCATSGFTFS DYMYWVRQTPKLEWVA YISNDSSAAYSDTVKG
 QVELVESGGGVVQPGRSLRLSCAASGFITS SYAMHWVRQAPGKGLEWVA VISYDGSNKYYADSVKG
 DVKLVESGGGVVQPGRSLKLSCATSGFTFS DYMYWVRQAPGKGLEWVA YISNDSSAAYSDRVKG
 * * * * *

74 75 82a 82b 83 84
 } } } } } CDR3
 RFTISTDNARTNLYLQMRLKSEDRAIYSCAR GLAWGAWFAY WQQGTLTVVSS
 56P1'CL RFTISRDNSKNTLYLQMNSLRAEDTAIYSCAR RSARTYYFDY WQQGTLTVVSS
 HumB3VH RFTISRDNSKNTLYLQMNSLRAEDTAIYSCAR TLAWTAWRAY WQQGTLTVVSS
 * * *

FIG. 11A.

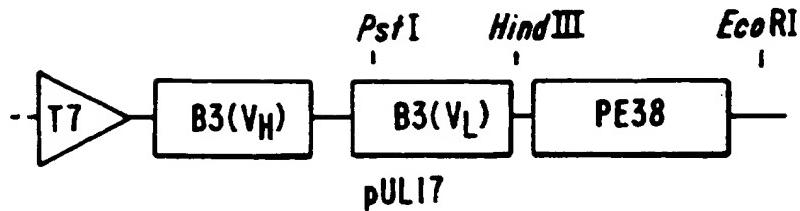
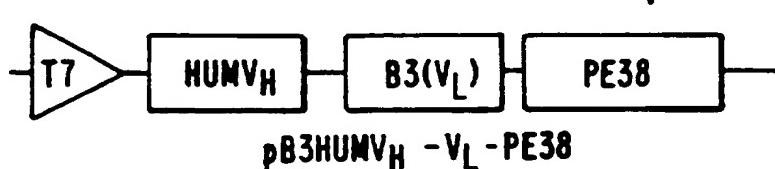
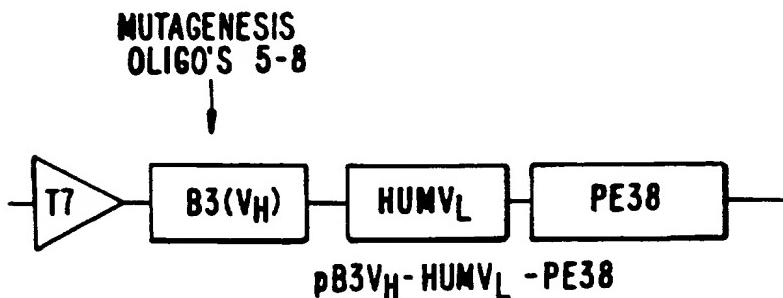
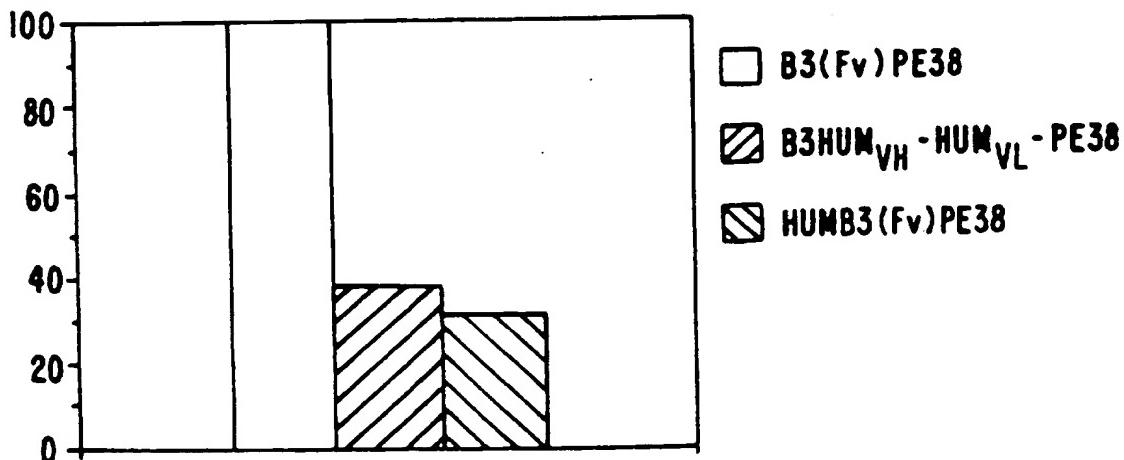
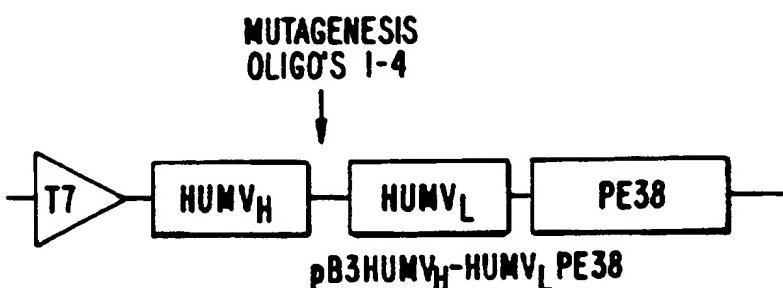
14 15 17 18 45
 || } } } CDR1 | CDR2
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 DIVMVTQSPLSPLPVTPGEPASIC RSSQSLHNSNGNYLD WYLOKPQQSPQLLIY LGSNRAS
 DVLMVTQSPLSPLPVTPGEPASIC RSSQIIVHNSNGNTYLE WYLOKPQSPQLLIY KVSNRFS
 * * *

83 100 104
 | |
 CDR3
 GVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC FQFSHPVFT FGSGTKLEIK
 GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC MOGLQTPOT FGQGTTKVEIK
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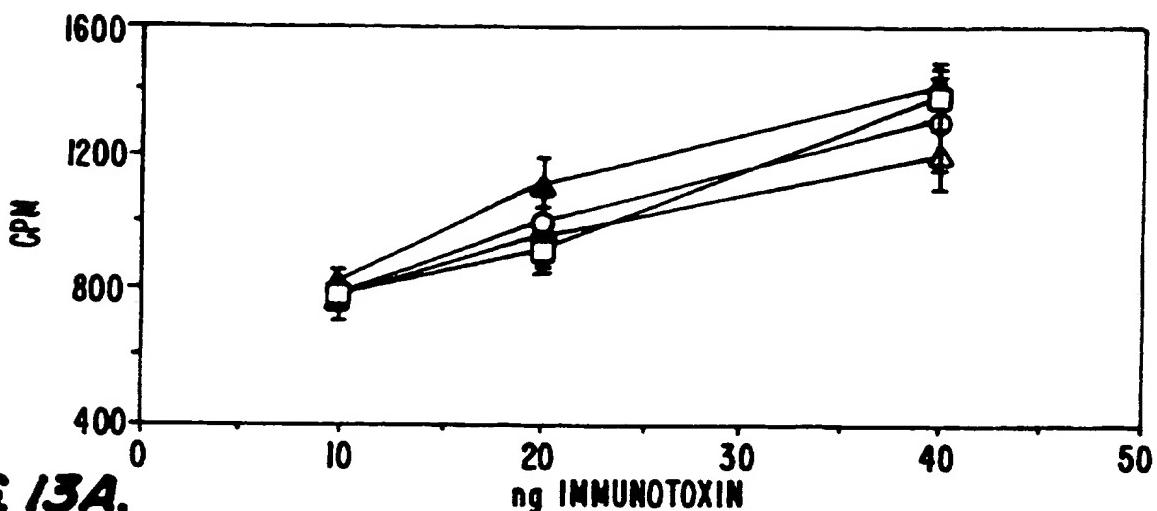
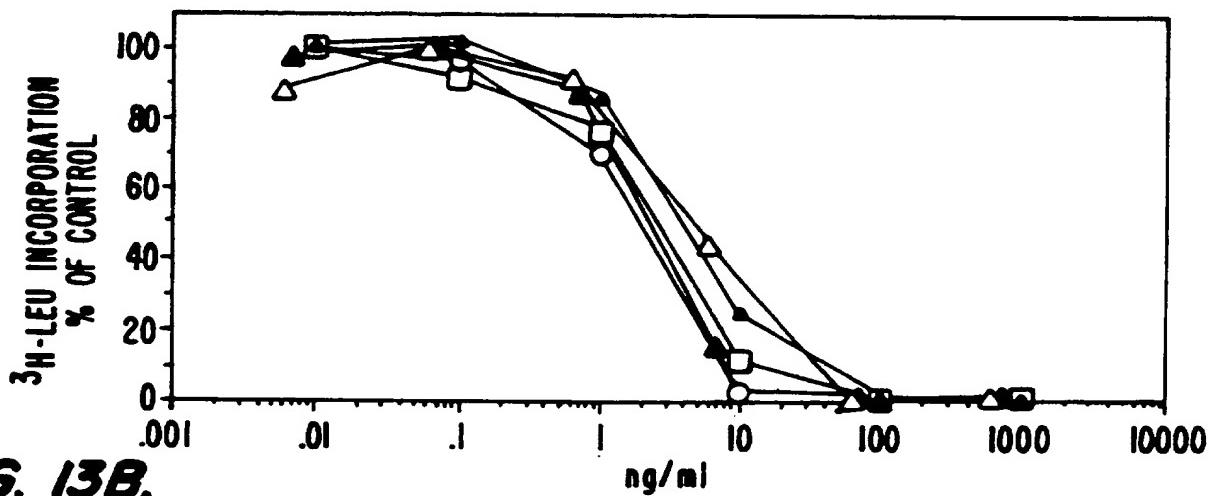
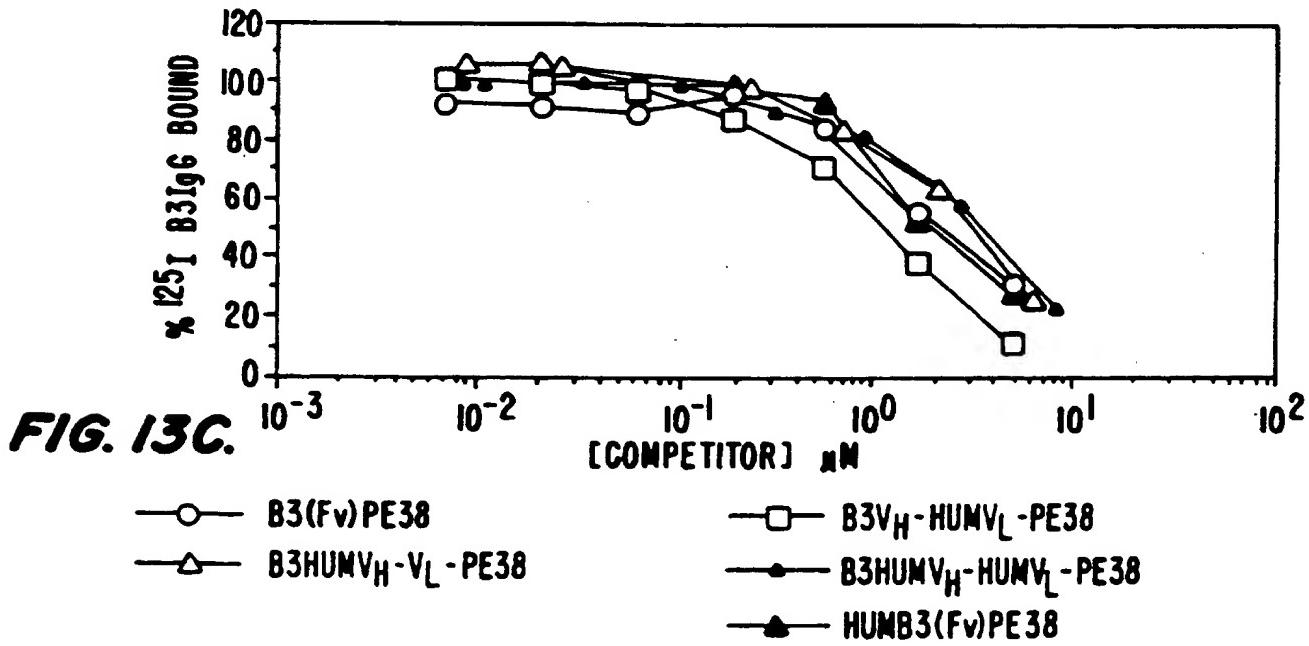
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FIG. 11B.

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FIG. 12A.**FIG. 12B.****FIG. 12C.****FIG. 12D.****FIG. 14.**

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**FIG. 13A.****FIG. 13B.****FIG. 13C.**

- B3(Fv)PE38
- △— B3HUMV_H-V_L-PE38
- B3V_H-HUMV_L-PE38
- B3HUMV_H-HUMV_L-PE38
- ◆— HUMB3(Fv)PE38

SUBSTITUTE SHEET (RULE 26)

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GAGGTGCAGCTGGTGGAAATCTGGAGGAGGCTTAGTGAAGCCTGGAGGGTCCCTGAAA
 E V Q L V E S G G G L V K P G G S L K
E V Q L V E S G G G L V K P G G S L
CTCTCCTGTGCAGCCTCTGGATTCATTTCAGTGACAATTACATGTATTGGGTTCGC
L S C A A S G F I F S D N Y M Y W V R

CDR 1

CAGACTCCGGAGAAGAGGGCTGGAGTGGGTGCAACCATTAGTGATGGTGGCACTTAT
Q T P E K R L E W V A T I S D G G T Y

CDR 2

ATCGACTATTCAGACAGTGTGAAGGGCGATTACCATCTCCAGAGACAATGCCAAG
I D Y S D S V K G R F T I S R D N A K

AATAATCTGTACTTGCAAATGAGCAGTCTGAGGTCTGAGGACACAGGCATGTATTAT
N N L Y L Q M S S L R S E D T G M Y Y

TGTGGAAGGAGTCCGATCTACTATGATTACGCCCGTTACTTACTGGGCCAAGGG
C G R S P I Y Y D Y A P F T Y W G Q G

CDR 3

ACTCTGGTCACTGTCTGCAGCCAAACGACACCCCCATCTGTCTATCCACTGGCC
C L V T V S A A K T T P P S V Y P L A

CCTGGATCTGCT

~~P G S A~~**FIG. 15A.**

GATGTTGTGATGACCCAGACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCC
D V V M T Q T P L S L P V S L G D Q A
D V V M T Q T P L S L P V S L G D
TCCATCTCTTGAGATCTAGTCAAAACCTTGTACACAGTGATGGAAAAACCTATT
S I S C R S S O N L V H S D G K T Y L

CDR 1

CATTGGTTCTGCAGAACGCTGGCCAGTCTCCAACGCTCCTGATCTACAAAGTTCC
H W F L Q K P G Q S P T L L I Y K V S

CDR 2

AACCGATTCTGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATT
N R F S G V P D R F S G S G S G T D F

ATACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGAGTTATTCTGCTCTCAA
I L K I S R V E A E D L G V Y F C S O

AGTACACATGTTCCGCTCACGTTGGTGTGGGACCAAGCTGGAGCTGAAACGGGCT
S T H V P L T F G A G T K L E L K R A

CDR 3

GATGCTGCACCAACTGTATCCATCTTCCCACCA

~~D A A P T V S I F P P~~

FIG. 15B.

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GAGGTGAAGCTGGTGGAAATCTGGAGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAAA
 E V K L V E S G G G L V Q P G G S L K
E V K L V E S G G G L V Q P G
CTCTCCTGTGCAACCTCTGGATTACTTCAGTGACTATTACATGTATTGGGTTCGC
L S C A T S G F T F S D Y Y M Y W V R
CDR 1
CAGACTCCAGAGAAGAGGGCTGGAGTGGGTCGCATACATTAGTAATGGTGGTAGC
Q T P E K R L E W V A Y I S N G G G S
CDR 2
ACCTATTATCCAGACACTGTAAAGGGCCGATTCAACCATCTCCAGAGACAACGCCAAG
T Y Y P D T V K G R F T I D R D N A K
AACACCCTGTACCTGCAGATGAGCCGTCTGAAGTCTGAGGACACAGCCATGTATTAC
N T L Y L Q M S R L K S E D T A M Y Y
TGTGCAAGGGGGCTCTGTGATGGTTCTGGTTGCTTACTGGGGCCAAGGGACTCTG
C A R G L S D G S W F A Y W G Q G T L
CDR 3
GTCACTGTCTCCTCAGGCGGAGGCAGGATCCGGT
V T V S S E C C G S C

FIG. 16A.

GATGTTTGTGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTGGAGATCAAGCC
D V L L T Q T P L S L P V S L G D Q A
D V L L T Q T P L S L P V S L
TCTATTCTTGTAGATCTAGTCAGAGCATTGTACATAGTAATGGAAACACCTATTAA
S I S C R S S O S I V H S N G N T Y L
CDR 1
GAATGGTACCTGCAGAAACCAGGCCAGTCTCAAAGCTCCTGATCTACAAAGTTCC
E W Y L Q K P G Q S P K L L I Y K V S
CDR 2
AACCGATTCTGGGTCCCAGACAGGTTAGTGGCAGTGGATCAGGGACAGATTTC
N R F S G V P D R F S G S G S G T D F
ACACTCAAGATCAGCAGAGTGGAGGCTFAGGATCTGGAGTTATTACTGCTTCAA
T L K I S R V E A E D L G V Y Y C E O
GGTCACATGTTCCATTACGTTGGCTCGGGACAAAGTTGGAAATAAGCGGGCT
G S H V P F T F G S G T K L E I K R A
CDR 3
GATGCTGCACCAACTGTATCCATCTTCCCCACCA
D A A P T V S I F P P

FIG. 16B.

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VH:
 DVKLVESGGGLVQPGGSLKLSCATSGFTS (DYYMY) WVRQTPEKRLIEWVA (YISNDSSAAYSDTVKG)
 RFTISTDNARNTLYLQMSRLKSEDTAIYSCAR (GLAWGAWFAY) WGQGTILVTVSS

LINKER:
 GGGGGGGGGGGGGGS

VL:
 DVLMTQSPLSLPVSLGDQASISC (RSSQIIVHSNGNTYLE) WYLQKPQSPKLLIY (KVSNRFS)
 GVPDRFSSFSFTDFTLKISRVEAEDLGVYYC (FQGSHVPFT) FGSGTKLEIK

FIG. 17.

VH:
 GATGTGAAGCTGGAGCTGGGGAGCGCTCGTAGCCCCGGCTCCCTGAAACTCTCCCTGTGCAACCTCTG
 GATTCACTTCAGTGAATATTACATGTATGGGTTGCCAGGGGGCAAGGGCCATACAT
 TAGTAATGATGATACTGGCCGGTTATTAGACACTGTAAAGGGCCGGTTACCATCTCTAGAGACAAAGCAAG
 AACACCCCTACTTGCAAAATGAACCCGCTGGCGGCCAGACAGCCATATTCCTGTGCAAAGGGACTGGCCT
 GGGGAGCCTGGTTGCTTACTGGGCCAAGGGACTCTGGTCACTGTCTCCTCA

LINKER: ggcggaggcgatccggtgggtggcgatctggagggtggcgaaagc

VL:
 GATGTGCTGATGACCCAGTCTCCATTGAGTTACCTGTCACCCCGGAGGCCCTCCATCTCTTGAGATCTA
 GTCAGATCATTTGACATAGTAATGGAAACACCTATTAGATGGTACCTGCAAGAACCCAGGCCAGTCTCCACAGCT
 GCTGATCTACAAAGTTCCAACCGATTCTGGGTCCAGACAGGTTCACTGGCAGTGGATCAGGGACAGATTTC
 ACACTCAAGATCAGCAGACTGGGAGTGGAGCTGAGGACGTTCAAGGGTTTATTACTGCTTCAAGGGTCACTGTCCATTCA
 CGTTCGGCCAGGGTACCAAGGGTCAAAGGGTCAAATAAA

FIG. 18.

INTERNATIONAL SEARCH REPORT

International Application No
US 95/13811

A. CLASSIFICATION OF SUBJECT MATTER	
IPC 6	C12N15/62 C12N15/13 C07K19/00 C07K16/30 A61K47/42
	A61K39/395 G01N33/577 G01N33/574 C07K16/00 C07K16/46
	//C07K14/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 07286 (THE UNITED STATES OF AMERICA) 15 April 1993 cited in the application see the whole document	1-30
X	BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1198, 1994 AMSTERDAM, THE NETHERLANDS, pages 27-45, XP 000564482 U. BRINKMANN ET AL. 'Immunotoxins against cancer' cited in the application see the whole document	1-30

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

- 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- 'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- 'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- '&' document member of the same patent family

Date of the actual completion of the international search

28 February 1996

Date of mailing of the international search report

25 MARCH 1996

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Nooij, F

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US95/13811

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 88, no. 19, 1 October 1991 WASHINGTON, DC, USA, pages 8616-8620, U. BRINKMANN ET AL. 'B3(Fv)-PE38KDEL, a single-chain immunotoxin that causes complete regression of a human carcinoma in mice.' cited in the application see abstract see figures 1,2 ---	1-30
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 89, no. 7, 1 April 1992 WASHINGTON, DC, USA, pages 3075-3079, XP 000264181 U. BRINKMANN ET AL. 'Independent domain folding of Pseudomonas exotoxin and single-chain immunotoxins: Influence of interdomain connections.' cited in the application see figure 1 ---	1-22
X	BIOCONJUGATE CHEMISTRY, vol. 5, no. 4, July 1994 WASHINGTON, DC, USA, pages 321-326, XP 000564453 I. BENHAR ET AL. 'Mutations of two lysine residues in the CDR loops of a recombinant immunotoxin that reduce its sensitivity to chemical derivatization.' cited in the application see abstract see figure 1 ---	1-30, 34-37
X	CANCER RESEARCH, vol. 53, no. 2, 15 January 1993 PHILADELPHIA, PA, USA, pages 334-339, P. FRIEDMAN ET AL. 'BR96 sFv-PE40, a potent single-chain immunotoxin that selectively kills carcinoma cells.' see abstract see discussion ---	1-30
X	THE JOURNAL OF IMMUNOLOGY, vol. 152, no. 5, 1 March 1994 BALTIMORE, MD, USA, pages 2377-2384, C. SIEGALL ET AL. 'In vitro and in vivo characterization of BR96 sFv-PE40.' see abstract ---	1-30
1		-/-

INTERNATIONAL SEARCH REPORT

International Application No
US 95/13811

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 90, no. 16, 15 August 1993 WASHINGTON, DC, USA, pages 7538-7542, U. BRINKMANN ET AL. 'A recombinant immunotoxin containing a disulfide-stabilized Fv fragment.' cited in the application see abstract ---	1-30
A	NATURE, vol. 309, no. 5963, 3 May 1984 LONDON, GB, pages 73-76, H. KLOBECK ET AL. 'Contribution of human VκII germ-line genes to light-chain diversity.' cited in the application see figure 2 ---	1-22, 34-61, 65-88
A	THE JOURNAL OF IMMUNOLOGY, vol. 150, no. 7, 1 April 1993 BALTIMORE, MD, USA, pages 2774-2782, U. BRINKMANN ET AL. 'Recombinant immunotoxins containing the VH or VL domain of monoclonal antibody B3 fused to Pseudomonas exotoxin.' see abstract ---	1-30
A	CANCER RESEARCH, vol. 54, no. 12, 1 July 1994 PHILADELPHIA, PA, USA, pages 3460-3467, M. CHOE ET AL. 'B3(Fab)-PE38M: A recombinant immunotoxin in which a mutant form of Pseudomonas exotoxin is fused to the Fab fragment of monoclonal antibody B3.' see abstract see figure 2 ---	1-30
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 91, no. 25, 6 December 1994 WASHINGTON, DC, USA, pages 12051-12055, I. BENHAR ET AL. 'Rapid humanization of the Fv of monoclonal antibody B3 by using framework exchange of the recombinant immunotoxin B3(Fv)-PE38.' see abstract ---	1-22, 65-88
		-/-

INTERNATIONAL SEARCH REPORT

Inventor Application No.
PCT/US 95/13811

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>PROTEIN ENGINEERING, vol. 7, no. 12, December 1994 OXFORD, GB, pages 1509-1515, XP 000482989 I. BENHAR ET AL. 'Cloning, expression and characterization of the Fv fragments of the anti-carbohydrate mAbs B1 and B5 as single-chain immunotoxins.' see abstract</p> <p>---</p>	1-22
P,X	<p>CLINICAL CANCER RESEARCH, vol. 1, no. 9, September 1995 PHILADELPHIA, PA, USA, pages 1023-1029, I. BENHAR ET AL. 'Characterization of B1(Fv)PE38 and B1(dsFv)PE38: Single-chain and disulfide-stabilized Fv immunotoxins with increased activity that cause complete remissions of established human carcinoma xenografts in nude mice.' see abstract</p> <p>---</p>	1-30
P,X	<p>THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 40, 6 October 1995 BALTIMORE, MD, USA, pages 23373-23380, I. BENHAR ET AL. 'Identification of residues that stabilize the single-chain Fv of monoclonal antibodies B3.' see the whole document</p> <p>-----</p>	1-30, 34-64

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/13811

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 30, 64, 94

because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 30, 64 and 94 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compound/composition.

2. Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 95/13811

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9307286	15-04-93	AU-B- 2779892 CA-A- 2120153 EP-A- 0610286 JP-T- 7502643	03-05-93 15-04-93 17-08-94 23-03-95

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